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THE VALUE OF EXFOLIATIVE CYTOLOGY IN THE DETECTION OF MALIGNANCY OF THE FEMALE GENITAL TRACT, WITH PARTICULAR REFERENCE TO THE SIGNIFICANCE OF BORDERLINE LESIONS*

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The value of exfoliative cytology in the early detection of malignancy of the female genital tract is generally accepted today. Malignant lesions which are not clinically suspicious can be detected by cytology at a stage when they are 99 - 100% curable, and cancer of the cervix may thus rightly be considered a preventable disease.[1]

The basis for discussion of any new investigation would appear to be:

1. Has the investigation so far proved its worth?
2. What is the accuracy that may be expected from the test?

HAS THE INVESTIGATION PROVED ITS WORTH?

Statistics of mortality rates from cancer of the cervix in centres where intensive cytological programmes have been conducted, show remarkable improvement.

In New York City the overall incidence of cancer of the uterus (cervix and body) rose slightly between the years 1942 and 1958, being 39·41 per 100,000 female population in 1942 and 41·52 in 1958. The mortality, however, dropped by nearly 50% (from 29·7 to 15·78).[2]

In British Columbia, where one-third of the women over the age of 20 have been cytologically screened, the incidence of infiltrating carcinoma of the cervix has dropped from 28·4 per 100,000 female population in 1955 to 19·7 in 1960.[3]

The improvement shown in figures from these areas does not appear to have been paralleled in surrounding areas where cytology has not been extensively practised. The improvement seems to be due mainly to the detection of *in situ* carcinoma, since the overall incidence of cervical cancer remains much the same while the fall has occurred in the incidence of infiltrating carcinoma. This is borne out by the rise in *in situ*, compared to invasive carcinoma during the last few years:

FREQUENCY OF *in situ* CANCER OF CERVIX

New York State general hospitals[2]	1946 — 3%; 1958 — 22%
Roswell Park Cancer Hospital[2]	1946 — 8%; 1958 — 15%
Ohio General Hospital	1953 — 8%; 1960 — 36%
Karl Bremer Hospital:	
	1957 - 58 — Less than 1%; 1959 - 62 — 20%

Today no one questions the fact that carcinoma-*in-situ* is a potentially malignant lesion. Regressions undoubtedly do occur, but are unpredictable. In the absence of treatment it is generally considered that carcinoma-*in-situ* almost invariably progresses to invasive carcinoma.[4,5] However, complete removal of the malignant focus can result in regeneration of normal epithelium. In a young woman wishing to have further children, a cone biopsy, which entirely removes the malignant focus, may be considered if the patient can be followed up adequately with cytology. Otherwise a total hysterectomy is the treatment for cervical carcinoma-*in-situ*.

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ACCURACY OF VAGINAL CYTOLOGY

The following table gives figures showing the percentage accuracy of vaginal cytology in various centres:

	Cases No.	*Cytology correct* No.	*Accuracy* %
Malignant disease of the female genital tract[6]	662	576	87
Non-malignant disease of the female genital tract[6]	137,138	136,932	99·8
Endometrial carcinoma[7]	63	40	63·5
Ovarian carcinoma[8]	119	48	40

Accuracy at Karl Bremer Hospital

Between June 1959 and December 1962 a total of 10,308 patients was examined; more than half this number were screened during 1962. The following table shows the percentage accuracy of vaginal cytology in this series:

	Cases No.	*Cytology correct* No.	*Accuracy* %
Non-malignant disease of the female genital tract[6]	10,165	10,035	98·7
Carcinoma of the cervix	103	100	97
Adenocarcinoma of the endometrium	18	11	61·1
Adenocarcinoma of the cervix	6	6	100
Sarcoma	4	1	25
Carcinoma of the ovary	12	8	66·6

Proved false negatives: 17 patients (class 3 is counted as negative).
Proved false positives: 130 patients (classes 4 and 5 are counted as positive).

The histogram shows that the number of false positives has fallen markedly since the opening of this laboratory (p. 920)

Papanicolaou's system of cytological classification, with slight adaptations, is used in the Karl Bremer Hospital laboratory. It is as follows:

Class 1: Normal.
Class 2: Benign atypical inflammatory cells.
Class 3 minus: Atypical cells, probably inflammatory.
Class 3 plus: Marked atypia of cells.
Class 4: Highly suggestive of malignancy.
Class 5: Cytologically malignant.

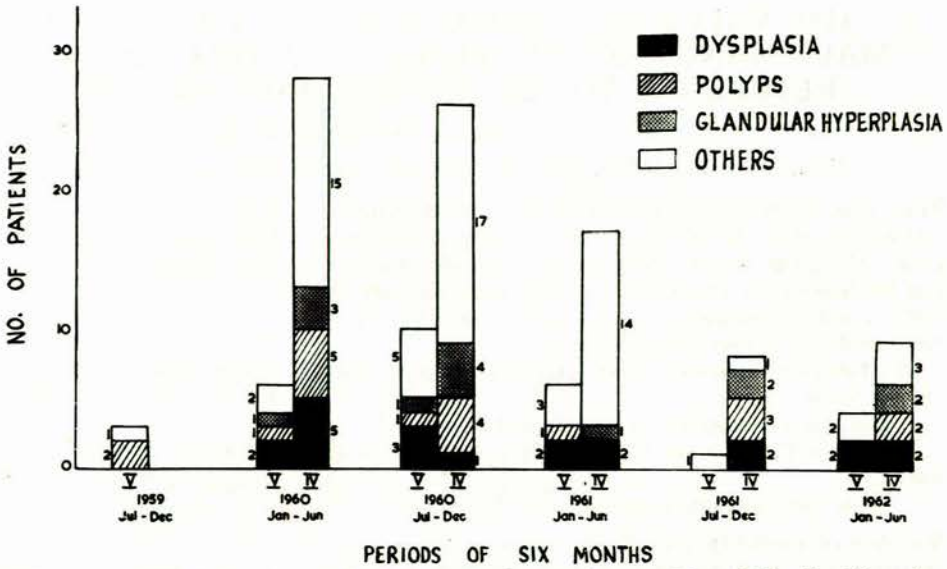
N.B.: Class 3 is not suspicious of malignancy in this laboratory, but we consider follow-up is essential. A biopsy is not cytologically indicated in class 3 unless specifically requested. The splitting of class 3 into minus and plus sections has been found to be of value to the clinicians.

DISCUSSION

It appears from the figures given above that cytological screening in respect of carcinoma of the cervix is extremely

reliable. It is also of limited value in the detection of malignant disease elsewhere.

It seems clear, therefore, that the field of cytology must be widened, and that more women should be screened in order to detect more patients with early cervical cancer. It should become a free, Government-sponsored, routine investigation, since it is an investigation which can lower the mortality from a preventable disease. In fact, it should become an integral and routine part of every gynaecological examination.



Histogram. Showing number of false-positive cases (up to July 1962). V=Class 5 (cytologically malignant) not histologically proved. IV=Class 4 (cytologically highly suggestive of malignancy) not histologically proved.

Since this desirable state of affairs has not yet come about, the following tentative suggestions are made to those who do not do cytology, be they specialists or general practitioners. I must emphasize that these suggestions are indeed a poor substitute for routine cytology, and that the majority of *in situ* carcinoma will be missed. Cytology smears should be taken:

1. From all patients who have a cervicitis or an erosion which does not respond to treatment. If the lesion looks suspicious a biopsy is naturally indicated. Cytology is never a substitute for histology.

2. From all cervices when there is an element of suspicion of malignancy and an immediate biopsy is for any reason impracticable.

3. From all women with cancer phobia.

4. From all patients with post-menopausal bleeding in whom the cause is apparently benign and in whom a dilatation and curettage and/or biopsy are not clinically indicated.

5. From all patients over 40 years of age.

New Method of Fixing and Dispatching Smears

In the past cytology smears have been fixed in a mixture of equal parts of ether and 95% alcohol immediately they are made. 'Cytodrifix' (Paragon) is now obtainable. A couple of drops of this on the wet smear replaces the ether-alcohol fixative. The slides are then immediately ready for mailing in flat cardboard boxes, which fit into a conventional envelope.

DIFFICULTIES OF DIAGNOSIS

Squamous Carcinoma of the Cervix

As can be seen from the foregoing statistics, the cytological accuracy of detection of squamous carcinoma is more than 97% in most laboratories.

There are two pitfalls in the cytological detection of squamous carcinoma.

1. In advanced fungating or ulcerative lesions the smear may be negative. This is due to the marked cell degeneration in a usually very bloody smear. Since these lesions are usually clinically obvious and would in any case be biopsied, they do not present a drawback to cytology other than from a statistical viewpoint.

2. *Borderline lesions of the squamous epithelium* of the cervix, which will be discussed hereunder, present an interesting problem both cytologically and pathologically.[9]

While carcinoma-*in-situ* cannot be considered a borderline lesion, its percentage incidence varies from 0·2% to 3·5% in different institutions. This is hardly a regional variation, and is almost certainly dependent on the different histological interpretations and the criteria which patho-

Fig. 1. Cytology of a patient with carcinoma-*in-situ*. This shows a group of pleomorphic nuclei with no individual cellular borders. The large irregular nucleus in the top left-hand corner shows irregular chromatin distribution characteristic of malignancy. (×250)

Fig. 2. Histology from same patient to whom Fig. 1 refers, showing the typical picture of carcinoma-*in-situ*. (×250)

Fig. 3. Irregular pleomorphic nuclei showing chromatin distribution suggestive of malignancy—class 4. (×250)

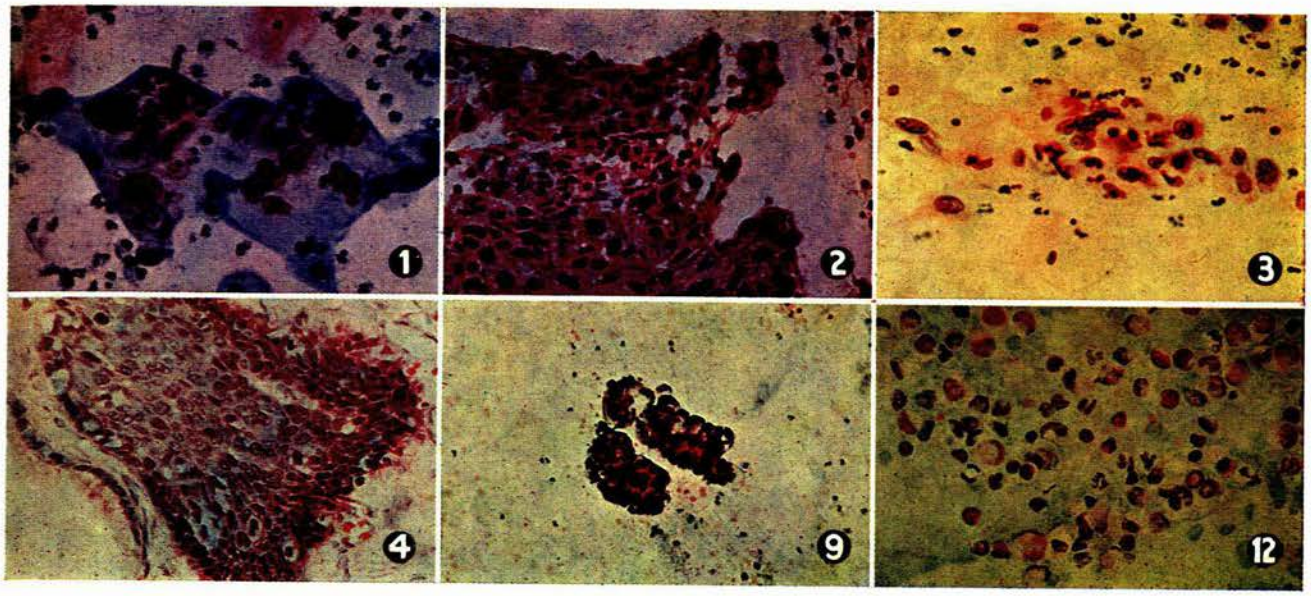
Fig. 4. Histology from same patient to whom Fig. 3 refers. Shows an area of marked epidermization of a gland. The irregular disposition of cells and the mitotic figures are visible. This may be considered a borderline lesion. (×250)

Fig. 5. Case 1. Class 4. Before cone biopsy. Histology at this stage negative for malignancy. (×800)

Fig. 6. Case 1. Class 4. 3 months after cone biopsy. Subsequent hysterectomy showed micro-invasive carcinoma. (×500)

Fig. 9. Adenocarcinomas may exfoliate large clumps of cells as seen in this picture. This is not usual and exfoliation is usually poor. (×80)

Fig. 12. A group of very degenerate cells, showing marked pleomorphism and chromatin variation. Class 4. This patient had a papillary cyst adenocarcinoma of the ovary. (×250)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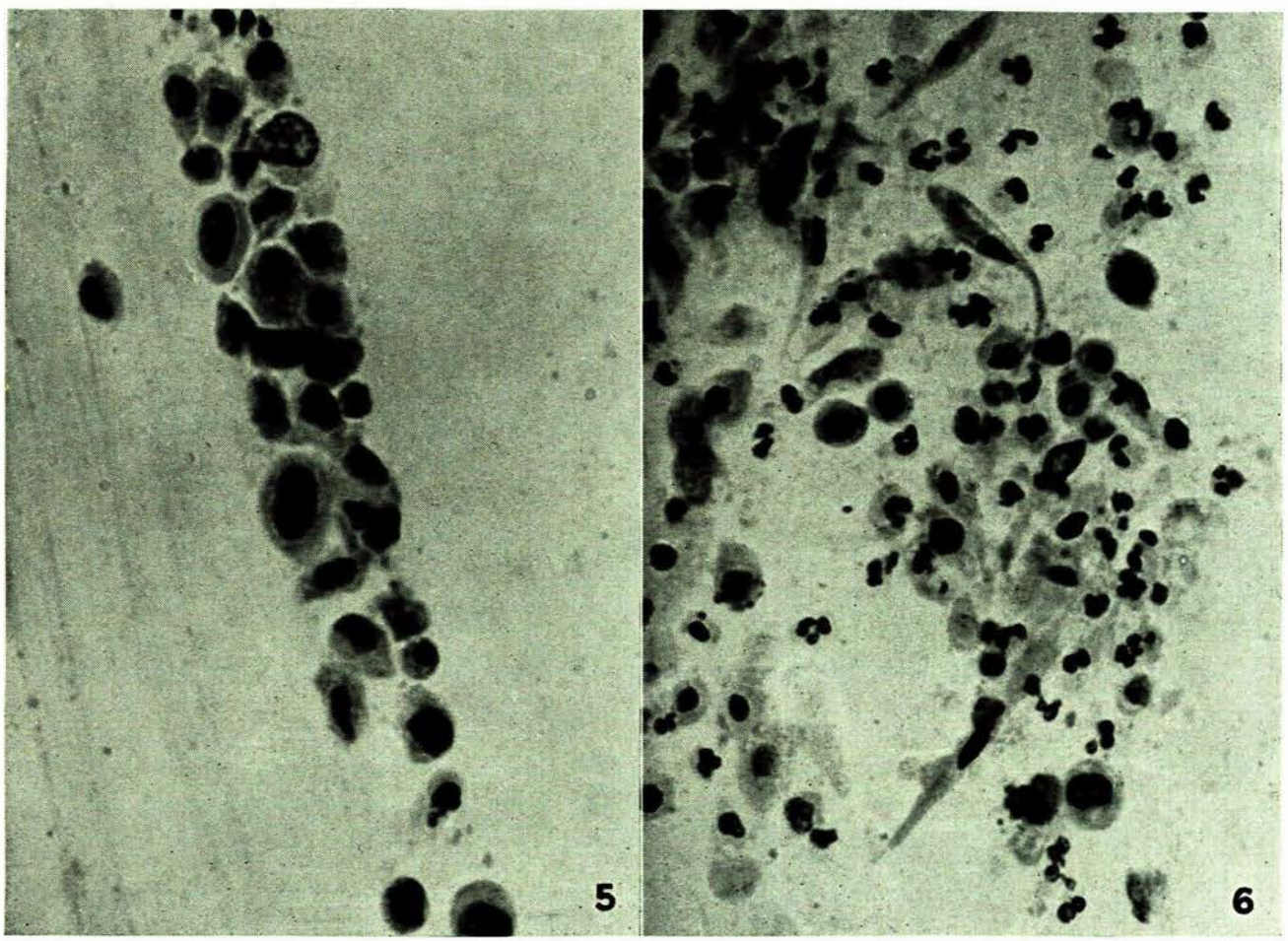
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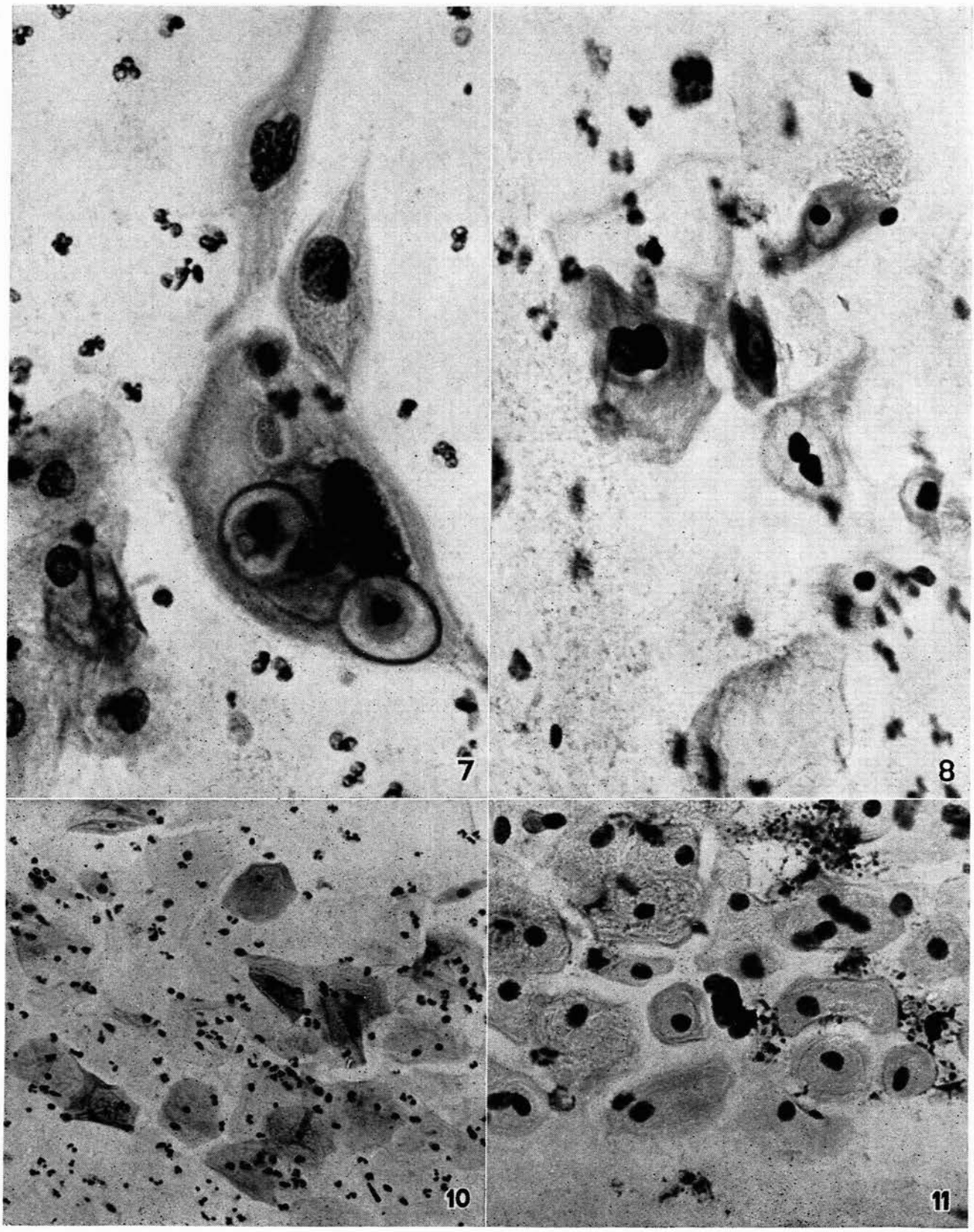
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logists apply for this diagnosis. In a survey in the USA identical sections from 20 patients were sent to 25 leading pathologists. The lesions in the sections were regarded as varying from a benign cervicitis to a carcinoma-*in-situ* with possible infiltration. The difference of opinion on these lesions was marked and 'could not be considered within desirable range'.[10] In another survey[11] 20% of the slides examined by 22 pathologists were placed in the group 'borderline — diagnosis uncertain'.

I must stress that a histological diagnosis of carcinoma-*in-situ* cannot be made from one or two sections. In all cases where cytology is suspicious for malignant squamous cells an adequate cone biopsy, which contains both endo- and ectocervix, should be removed in one piece. The cut surface should be painted with 1% silver nitrate so that the edge of the section can be studied to decide if removal has been complete. The cytology findings should be reported to the pathologist.

It is generally considered that, apart from research purposes, serial sections of the specimen are unnecessary. In a recent symposium[5] by pathologists in countries as widely scattered as Peru, the USA, England, Germany, France and Australia, it was generally agreed that all cone biopsies should be sectioned radially into 6 - 8 blocks which should include endo- and ectocervix. Three to four sections from each block at different levels should be made. This will detect the average carcinoma-*in-situ* and is considered a practical procedure in any laboratory. It emphasized that a diagnosis of carcinoma-*in-situ* can never be made or excluded on one histological section or on serial sections of one isolated biopsy or section of a biopsy. Another point is that carcinoma-*in-situ* has a characteristic tendency to strip from the underlying stroma. This epithelium, therefore, may not be included in the section. It is felt that vigorous swabbing at operation may be a factor in this stripping.

The histological interpretation of any borderline lesion in the best hands is liable to vary. Figs. 1 and 2 show the cytology and histology, respectively, from a patient with a classical carcinoma-*in-situ*. Figs. 3 and 4 show the cytology and histology, respectively, of what may be described as a borderline lesion and may be open to various interpretations.

This brings up the interesting question of how many of these cervical lesions which exfoliate atypical cells are in point of fact pre-malignant, i.e. just one step behind a carcinoma-*in-situ*—the case that the pathologist had difficulty in deciding about. One may take a step further back and speculate on what proportion of metaplasia, dysplasia and basal-cell hyperplasia may progress to malignancy. It is interesting to note from the histogram that a high proportion of false-positive cytological reports have shown pathological changes which, collectively, can be described as more significant than an inflammatory change *per se*.

The following case histories illustrate these so-called borderline lesions:

Case 1

European, aged 50 years.

29 October 1962: Attended the outpatient department complaining of pruritus vulvae and a discharge. *Cytology*—class 3 minus. Marked monilial infection with a minimal squamous-cell atypia.

18 January 1963: Attended the outpatient department because of two episodes of intermenstrual bleeding. *Cytology* — class 3 plus. Request for further smears immediately.

30 January: Cytology — class 4, highly suspicious of malignancy (Fig. 5). Biopsy necessary. *Histology*—chronic cervicitis.

20 April: Attended the outpatient department in reply to a request to come for cytological follow-up. *Cytology* — still class 4 (Fig. 6). Further investigation necessary.

(Subsequent to the presentation of this paper on 24 April, the patient had a hysterectomy on 3 May, and was found to have a squamous carcinoma with minimal infiltration, situated deep in the endocervix in an area of epidermization of glands).

Case 2

Non-European, aged 33 years.

19 October 1962: Attended the outpatient department complaining of pain in the lower abdomen. Acute pelvic infection was diagnosed. *Cytology* — class 3 minus. Atypia probably from trichomonas infection.

28 November: Attended outpatient department in response to a follow-up letter. *Cytology* — class 4 (Fig. 7). Biopsy necessary. The trichomonas infection had cleared, but a marked cell atypia was still present.

10 December: A biopsy was taken. *Histology*—chronic cervicitis with epidermization of glands and atypia of squamous epithelium.

Case 3

European, aged 62 years.

15 August 1962: Attended the outpatient department complaining of a vaginal discharge. *Cytology* — class 3 plus. Trichomonas infection, squamous-cell atypia.

18 November: Attended outpatient department for follow-up. *Cytology* — class 4 (Fig. 8). Inflammation cleared. The smear suggested that a very high oestrogen level was present (this point will be discussed later). Biopsy and dilatation and curettage necessary.

15 December: Admitted. No inflammatory signs clinically or cytologically, but a squamous-cell atypia was still present cytologically. *Histology*—'Erosion of the cervix with a chronic cervicitis. A slight atypia of squamous epithelium is due to inflammatory changes.'

In the past the tendency has been to consider the cytologist's interpretation wrong when class 4 or 5 smears have had a negative histology report for malignancy. The tendency is to discharge the patient and discredit the cytological findings.

There is no doubt that a high proportion of squamous-cell atypias do regress. The present difficulty is that we do not know which of these borderline lesions will progress to malignancy and which will not. We feel that it is only through routine follow-up of patients with class 3,

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Fig. 7. Case 2. Class 4. Note marked anisocytosis. Lower large cell shows inclusion bodies and increased chromatin in nucleus. Two upper cells show irregularity of chromatin distribution and nuclear margin. (×1,000)

Fig. 8. Case 3. Class 4. Note the marked squamous-cell atypia. In addition, cornified cells are present, suggesting a high oestrogen level. Age, 62. (×600)

Fig. 10. Shows highly cornified cells in a patient aged 77 years, who had an adenocarcinoma. No malignant cells were found in these smears. (×200)

Fig. 11. While a highly cornified cell picture is more usual in patients with adenocarcinoma, an atrophic smear may be present, as seen in this picture, where the only atypicality is the group of histiocytes shown in the centre of the field. (×450)

4 and 5 cells that we will get any nearer to sorting out the problems of what may be called pre-cancer. It is essential to realize that class 3, which in this laboratory amounts to 6% of the total slides examined, is not just a cytologist's escape hole. We admit that we cannot interpret these changes, but they are there nevertheless. The point is, can we ignore them?

Finally, it should be emphasized that cytology suggestive of malignancy (class 4 and 5), in which the histology is negative, should not be regarded as incorrect. Further follow-up is essential. The absence of correlation between cytology and histology may indeed be due to incorrect cytological interpretation, but it may also be due to an inadequate biopsy or inadequate sectioning of biopsy material.

Adenocarcinoma

As stated earlier in this paper, the cytological pick-up of malignant cells from an adenocarcinoma of the endometrium (Fig. 9) is much lower than from malignant lesions of the cervix. Various methods of improving the cell pick-up have been suggested, e.g. endometrial aspiration and the introduction of a small brush into the endometrial cavity. These have not been used in this country and a dilatation and curettage seem preferable.

Recently attention has been focused on various ancillary pointers which should cause the cytologist to suspect the presence of an adenocarcinoma, even in the absence of malignant or suspicious cells.[7] When these pointers are present we classify the patient in class 3 or even class 4, as a routine. The pointers are:

1. An appearance on cytological examination consistent with *a high oestrogen level* in a menopausal or post-menopausal patient. It is obviously imperative to know whether patients are receiving any type of oestrogen therapy. Fig. 10 shows the smear from a patient aged 77 years who had received no oestrogen therapy. The highly cornified picture, suggesting a high oestrogen level, is apparent. No malignant cells were present on the smears, but the patient had an adenocarcinoma of the uterus.

Unfortunately this pointer is by no means always present. Fig. 11 shows a normal atrophic smear from a woman who was proved histologically to have an adenocarcinoma of the uterus. It is interesting that this patient also had an ovarian tumour that was histologically highly suggestive of a Brenner tumour. This is so rarely associated with an adenocarcinoma of the corpus, in contrast to the granulosa-cell tumours, that the diagnosis was questioned. This atrophic-cell picture, which shows no evidence of a high oestrogen level, would be more in keeping with a Brenner tumour.

2. *The presence of histiocytes;* usually they are small with finely vacuolated cytoplasm. Fig. 11, from the above-mentioned patient with endometrial carcinoma, also shows a group of histiocytes found in this atrophic smear. In this case they were found retrospectively and the cytological report given was negative.

3. The presence of endometrial cells after the 10th day of the cycle.

These pointers are useful, but, as has been pointed out, may be absent or missed. Cytology should never be relied on to exclude intra-uterine pathology. A curettage is always necessary.

It is interesting to note that a high proportion of false-positive results from this laboratory, in cases where we have suspected an adenocarcinoma, have been from patients with either polypi or endometrial hyperplasia. It is also noteworthy that the slides of patients with adeno-carcinomas in which we have had false-negative results, with one exception, have all had one or more ancillary pointers, and have thus been classified in class 3 and have caused us to suggest a curettage.

Carcinoma of the Ovary

In a recent article, Graham and Van Niekerk, of Pretoria,[8] stated that a retrospective study of smears from patients with ovarian carcinoma showed that 40% of the patients exfoliated cells which were found in the vaginal smears. This was independent of whether the tumour was encapsulated, was confined to the ovary or had metastatic deposits. These cells are reported to have characteristic red nucleoli.

Our experience, which is naturally more limited, is that the cells which lie in loose groups as described, are usually degenerate and the morphology is indistinct and difficult to interpret. Fig. 12 shows cells which were considered suspicious of malignancy (class 4). It was found on laparotomy that the patient had an unsuspected papillary-cyst adenocarcinoma of the ovary.

Carcinoma of the Fallopian Tubes

The classical finding of malignant cells from a carcinoma of the tube 8 years ago by the late Dr. P. H. Oosterhagen, of Pretoria, probably represents the first cytological pick-up of carcinoma of the fallopian tube in South Africa.[12] There have been no such cases in this laboratory as yet.

SUMMARY

This paper deals with the value of exfoliative cytology in lowering the mortality of carcinoma of the cervix. The investigation is also worth while in the detection of malignancy of the corpus, ovaries and tubes, although it is not so accurate in cancer of these sites.

The importance of follow-up of atypical cells from possible borderline lesions is stressed. The value of adequate biopsies and examination of biopsy material is emphasized.

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